

Photorepair in Larval Anchovy
'Engraulis mordax'

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RESEARCH NOTE

PHOTOREPAIR IN LARVAL ANCHOVY, *ENGRAULIS MORDAX*

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Abstract—Photorepair of UV-B lesions occurred in embryonic northern anchovy larvae. The photoreactive fluence rate required to fully activate photorepair mechanisms was less than 10% of that available from the sun on a clear day in March (33N). Even with UV-B enhancement from ozone depletion, sufficient photoreactive fluence exists in the sea to ensure maximal photorepair of UV damage in anchovy larvae.

INTRODUCTION

Photorepair of UV-B induced lesions occurs in almost every species of animal and plant studied (Cleaver, 1974; Cook, 1970; Halldal and Taube, 1972). The existence and function of photoreactivating enzyme has been demonstrated in fish (Hart and Setlow, 1975; Hart *et al.*, 1977; Regan and Cook, 1967), and photorepair has been shown to have a mitigating influence on UV-B induced damage in developing amphibia (Blum *et al.*, 1957; Worrest and Kimeldorf, 1976). Our objectives were to verify the existence of photorepair of UV-B (286–320 nm) induced damage in larval anchovy, determine if photorepair can be expected to be fully activated in the sea under present and reduced ozone concentrations, and determine if photorepair was fully activated in our past UV experiments (Hunter *et al.*, 1979). These objectives are of

importance in the assessment of effects of ozone depletion on the survival of anchovy larvae in the sea.

MATERIALS AND METHODS

The eggs and yolk-sac larvae were reared in 1/ polypropylene beakers at 16°C. Each treatment consisted of 15 beakers, stocked with 50 eggs each. Eggs and larvae were exposed to four daily exposures of UV and visible light, and survival and growth assessed on the fifth day. The UV-B was provided by Westinghouse FS-40 sunlamps[®] filtered by 0.13 mm of cellulose triacetate (285 nm limit, Fig. 2) and the visible light by General Electric Chroma 50 lamps. Control containers were covered with Mylar[®] eliminating UV-B. UV-B was monitored continuously using a Norris UV-B radiometer (Optronics Model 725) and UV and visible light levels were calibrated with a spectroradiometer (Optronics Model 741-V). For further details of the apparatus, light sources, filters, and spectra, see Hunter *et al.* 1979.

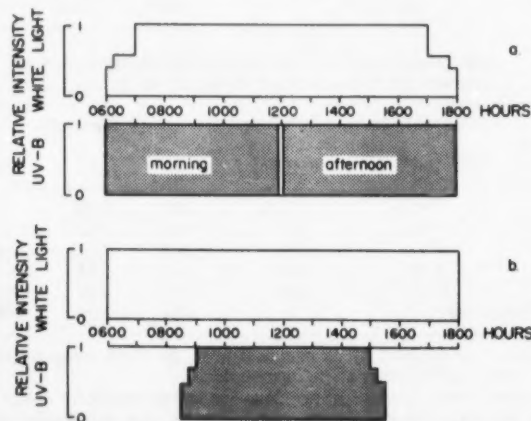


Figure 1. Schematic representation of the irradiation regime of UV-B (shaded area): (a) regime used to demonstrate photorepair (morning exposure compared to afternoon exposure); and (b) regime used to measure the effect of intensity of white-light energy on photorepair, with UV-B fluence constant.

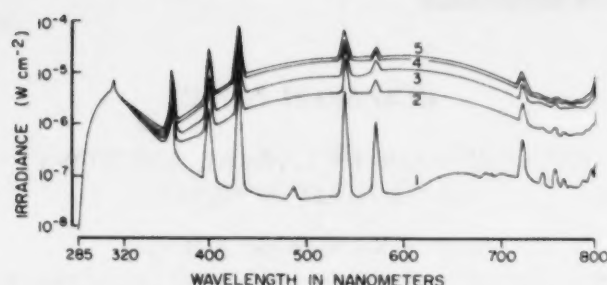


Figure 2. Spectral irradiance of the 5 different fluences used to measure the relation between photorepair energy and survival of embryonic northern anchovy. The numbers (1-5) refer to the chambers listed in Table 1.

To verify the existence of photorepair, we compared growth and survival of larval anchovy exposed to UV radiation over the first 6 h (morning) of a 12 h (white light) day to that of larvae exposed over the second 6 h (afternoon) (Fig. 1a). The effects of the morning and afternoon UV-B exposure were compared at six different UV-B fluences.

*Weighted dose using the DNA action spectrum of Setlow (1974) modified for anchovy mortality by Hunter *et al.* (to be published). Use of this modification of the DNA action spectrum does not imply that photorepair of DNA damage existed. The spectrum was selected using broad-band spectroscopy (see Hunter *et al.*, to be published) for details.

To determine the relation between the UV-induced mortality and the intensity of photorepair fluence, we partitioned water tables into five chambers and varied the photoreactive fluence within each chamber by varying the number of Chroma 50® lamps (Fig. 2). Each chamber received the same daily UV-B fluence ($189 \text{ Jm}^{-2} \text{ DNA eff.}^*$)

Table 1. Photorepair of UV damage to embryonic northern anchovy. Effect of temporal occurrence of 6 h UV-B fluence within 12 h white-light day.

Date	Cumulative UV-B fluence $\text{Jm}^{-2} \text{ DNA eff.}^*$	Timing of 6 h UV fluence	Survival†			Standard Length		
			$\bar{x} \pm s$	s	% of control	$\bar{x} \pm s$	s	N
3 May, 1979	ϕ	—	41.2	2.91	100.0	3.36	0.23	100
	661	morning	38.9	7.02	94.4	3.22	0.36	100
	683	afternoon	16.3	5.68	39.6	2.67	0.56	100
	947	morning	25.8	7.58	62.6	3.07	0.48	100
	977	afternoon	12.2	3.65	29.6	2.49	0.24	91
	1066	morning	32.7	6.93	79.4	2.80	0.47	100
2 June, 1979	1096	afternoon	16.9	7.90	41.0	2.60	0.36	100
	ϕ	—	44.5	4.17	100.0	3.47	0.27	100
	610	morning	42.1	2.83	94.7	3.59	0.26	100
	629	afternoon	22.1	4.40	49.7	3.45	0.56	100
	1221	morning	13.9	6.28	31.3	3.36	0.58	93
	1262	afternoon	6.1	4.20	13.7	2.29	0.38	51
	1812	morning	9.2	6.52	20.7	2.67	0.49	75
	1864	afternoon	3.3	2.58	7.4	2.33	0.30	41

Effect of daily dose of photorepair energy on survival at a constant UV-B fluence (daily fluence of $189 \text{ Jm}^{-2} \text{ DNA eff.}$)

Chamber number	Daily dose photorepair energy (kJm^{-2})		Survival†		
	(285-800 nm)	(320-500 nm)	$\bar{x}$	s	% of control
1	52.5	21.9	10.0	8.23	26.0
2	515	157	33.4	5.47	87.0
3	1170	310	34.8	3.65	90.6
4	2050	590	35.3	5.77	91.9
5	2130	600	37.5	4.56	97.7
Mylar® control§			38.4	3.20	100.0

*Dosage was weighted by an empirical fit to the DNA action spectrum of Setlow (1974) see Hunter *et al.* (to be published).

†Stocking density was 50 eggs per container, and means are for 15 containers.

‡All morning-afternoon pairs of treatments are statistically different ($P \leq 0.05$).

§Three control containers were in each of the five chambers. No differences existed in the mean survival among them.

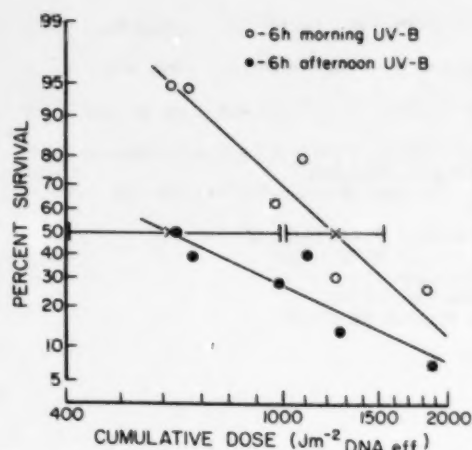


Figure 3. Survival of embryonic northern anchovy after 4 daily doses of UV-B when exposure to UV-B occurred in the morning (open circles) or afternoon (closed circles). The cumulative UV fluence (280–320 nm, log scale) is weighted by the DNA action spectrum of Setlow (1974) using the empirical fit of Hunter *et al.* (to be published), and the percent survival is on a probit scale (Finney, 1952). Crosses indicate LD_{50} and the bars are the 95% confidence intervals. (Morning exposure; $LD_{50} = 12535 \text{ Jm}^{-2} \text{ DNA eff.}$, 95% C.I. = 1019–1542. Afternoon exposure; $LD_{50} = 636 \text{ Jm}^{-2} \text{ DNA eff.}$, 95% C.I. = 413–978). Lines are the regression of survival probit, y , on log dose, x ; $y = 5.482 \times -11.984$ for morning, and $y = 2.724 \times -2.636$ for afternoon exposure.

and the 7 h UV-B period was centered in the 12 h white light day (Fig. 1b). The UV-B dose was equivalent to the daily dose just beneath sea surface on a clear late March or early April day at our latitude (33N).

RESULTS AND DISCUSSION

The LD_{50} dose (cumulative UV-B dose producing 50% mortality in 4 days) for larvae exposed to UV-B in the morning was about twice that for those exposed in the afternoon (Fig. 3). Thus, exposure to 6 h of white light (320–800 nm) immediately after a 6 h UV-B exposure greatly increased survival; this demonstrates the existence of photorepair in larval anchovy. In addition, larvae surviving the morning exposure were consistently larger at all dosages than were those surviving an afternoon exposure (mean standard length, Table 1). Not only does photorepair exist in larval anchovy, but its action translates directly into survival and growth.

The action spectrum for photorepair in anchovy is unknown. Consequently, the effect of photorepair fluence on larval survival is shown as a function of the integrated, unweighted photoreactive fluence (320–500 nm) in Fig. 4 and given for two band widths (320–500 nm; 285–800 nm) in Table 1. Survival of larvae after four daily UV-B exposures of

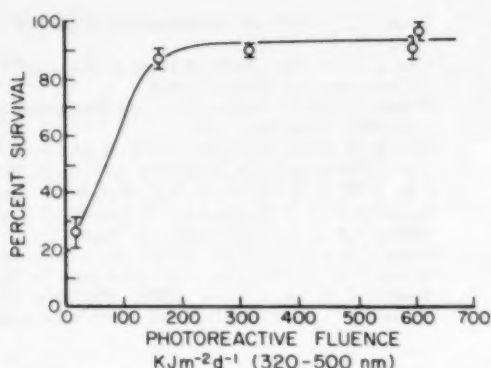


Figure 4. Survival of embryonic northern anchovy after 4 daily exposures to UV-B in relation to daily dosage of photoreactive fluence (320–500 nm in kJm^{-2}); daily UV-B fluence was constant at $189 \text{ Jm}^{-2} \text{ DNA eff.}$. Points are the mean survival of 15 containers (normalized to control) and bars are the 95% confidence intervals.

$189 \text{ Jm}^{-2} \text{ DNA eff.}$ increased sharply when the daily fluence of photorepair energy increased from 22 to 310 kJm^{-2} (320–500 nm). Thus, photorepair mechanisms appeared to be fully activated at daily fluences equal to or greater than 310 kJm^{-2} (320–500 nm) or 1170 kJm^{-2} (285–800 nm). As this is less than 60% of the fluence used in our past work (Hunter *et al.*, 1979; Hunter *et al.*, to be published), we conclude that photorepair occurred at maximal rates in those experiments.

The photorepair fluence necessary for maximal repair may vary with UV-B fluence rate. The UV fluence rate used here, $8.1 \text{ mWm}^{-2} \text{ DNA eff.}$ (daily fluence of $189 \text{ Jm}^{-2} \text{ DNA eff.}$), is relatively high, and lower photoreactive fluences may be adequate at lower UV-B fluence rates. In a single experiment, unlike the work described above, we were not able to detect an effect of shifting the period of UV-B exposure to the last 6 h of a 12 h (white light) day when the UV-B exposure was administered over 12 days at low fluence rates (1.8 and $2.8 \text{ mWm}^{-2} \text{ DNA eff.}$; daily fluence of 42 and $66 \text{ Jm}^{-2} \text{ DNA eff.}$). This indicates that photorepair may keep up with photorepairable damage at such low fluence rates.

Even at the relatively high UV-B fluence rates employed in the 4-day experiments, the photoreactive fluence (320–500 nm) needed to fully stimulate photorepair was about 10% of that available from the sun during a clear equinox day at our latitude (33N). Just beneath the water surface, the ratio of photoreactive fluence (320–500 nm) to UV-B fluence (290–320 nm) is about 100/1. This ratio will increase with depth in the ocean (Smith and Baker, 1979). Thus, photorepair will operate maximally at any depth even under the added stress of enhanced UV-B from ozone depletion.

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